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=> d his

     (FILE 'HOME' ENTERED AT 10:56:39 ON 05 FEB 2004)

     FILE 'HCAPLUS' ENTERED AT 10:57:10 ON 05 FEB 2004
L1          4185 S GLYCOSYLTRANSFERASE
L2           254 S L1 (L) (FUS? OR CHIMER? OR HYBRID?)
L3            30 S L2 (L) PREP/RL
L4            11 S L3 AND PD<19981119
L5             8 S L3 AND PD<19971119

     FILE 'STNGUIDE' ENTERED AT 11:03:05 ON 05 FEB 2004

     FILE 'HCAPLUS' ENTERED AT 11:06:33 ON 05 FEB 2004

     FILE 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT,
     CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DISSABS, DGENE,
     DRUGB, DRUGMONOG2, IMSDRUGNEWS, DRUGU, IMSRESEARCH, ..' ENTERED AT
     11:14:30 ON 05 FEB 2004
L6          3730 S L2
L7          2711 S L6 (L) (PREP/RL OR MAKE OR SYNTHE? OR FERMENT? OR PRODUC?)
L8          1061 S L7 (L) (SACCHARIDE OR OLIGOSACCHARIDE OR GLYCOLIPID)
L9           788 DUP REM L8 (273 DUPLICATES REMOVED)
L10          679 S L8 (L) (MICROORGANISM OR PLANT OR BACTER? OR EUBACTER?)
L11          632 DUP REM L10 (47 DUPLICATES REMOVED)
L12          410 S L10 (L) (NUCLEOTIDE) (L) SUGAR
L13           53 S L11 AND PY<1998
L14           78 S L11 AND PY<1999
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L14 ANSWER 4 OF 78 PROMT COPYRIGHT 2004 Gale Group on STN

ACCESSION NUMBER: 2000:171472 PROMT
TITLE: `TWO-IN-ONE' APPROACH ENZYME FUSION BETTER WAY OF MAKING CARBOHYDRATES.
SOURCE: BIOWORLD Today, (**18 Aug 1998**) Vol. 9, No. 158.
PUBLISHER: American Health Consultants, Inc.
DOCUMENT TYPE: Newsletter
LANGUAGE: English
WORD COUNT: 956
FULL TEXT IS AVAILABLE IN THE ALL FORMAT

AB "What can you infer about the Creator by studying his works?" J.B.S. Haldane was reportedly asked. "He has an inordinate fondness for beetles," answered the biologist, referring to the prevalence of the insects on the planet.

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L14 ANSWER 4 OF 78 PROMT COPYRIGHT 2004 Gale Group on STN

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"What can you infer about the Creator by studying his works?" J.B.S. Haldane was reportedly asked. "He has an inordinate fondness for beetles," answered the biologist, referring to the prevalence of the insects on the planet.

Today, someone thinking in molecular terms might cite "an inordinate fondness for carbohydrates." Once thought to be "hydrates of carbon" - a description later found to be inaccurate - carbohydrates are the most abundant organic compounds found in nature. They are made and eaten by nearly all ***plants*** and animals. Sugars, starches, cellulose, chitin, and many other cellular **products** are carbohydrates. They serve as energy sources, structural components and as part of DNA and RNA molecules. Streptomycin and some other antibiotics are carbohydrate derivatives.

But where are all the other carbohydrate-based drugs one might expect, given the widespread and important functions carbohydrates play in the body? Where are the carbohydrates for treating cancer, inflammatory and infectious diseases, and other disorders?

At least 17 companies are working on them. Most are in late stage development. A few are in the clinic.

The structural complexity of many carbohydrates presents a significant challenge to chemists. Several groups have developed new procedures designed to simplify the **synthesis** of complex carbohydrates. These approaches combine traditional methods of chemical **synthesis** and the use of enzymes.

"I think that the status of the **synthesis** technology is already adequate for high-value **products** like therapeutic and medical ***products.*** But there are important markets that can be addressed in a consumer **product** arena as well," said Jim Paulson, chief scientific officer and general manager of Glytec, a unit of San Diego-based Cytel Corp. that specializes in manufacturing carbohydrates for corporate partners.

In May, Cytel received a patent for a new way to enzymatically ***synthesize*** complex carbohydrates, also called **oligosaccharides**. Dubbed Sugar Nucleotide Cycling (SNC), this process uses recombinant ***glycosyltransferase*** enzymes to link up carbohydrate components in a way closer to that found in nature than in a traditional chemistry lab. The process allows Cytel to achieve yields nearing 100 percent for certain carbohydrates.

Now, Cytel scientists have teamed up with researchers from the Institute for Biological Sciences of the National Research Council, in Ottawa, Ontario, to provide the low-cost, high-yield technology needed to manufacture complex carbohydrate drugs and other commercial **products** in a commercially feasible way.

Warren Wakarchuk, a research officer at the Institute for Biological Sciences, and his co-authors describe their advance in "The **synthesis** of sialylated **oligosaccharides** using a CMP-Neu5Ac **synthetase** /sialyltransferase **fusion**," which appears in the August issue of Nature Biotechnology.

The researchers succeeded in combining two enzymes used in the SNC process into one molecule. The enzymes, sialyltransferase and a sugar- nucleotide ***synthetase*** , are essential for the large-scale enzymatic ***synthesis*** of certain carbohydrates called sialyated ***oligosaccharides.*** "Linking them together seemed to be a logical step, given that both of them express very well and that we were trying to avoid having to do two purification steps," Wakarchuk said.

Carbohydrates Made In Kilogram Quantities

The strategy of **fusing** proteins has been used before but never applied to the **synthesis** of **oligosaccharides**.

The custom-assembled, bifunctional enzyme was constructed using genes cloned from Neisseria meningitidis **bacteria**. The genes were inserted into Escherichia coli **bacteria**, which **produced** large amounts of the **fusion** protein. Importantly, the overexpressed **product** could be purified using a relatively simple procedure. **Bacterial** enzymes of this type have been easier to **produce** on a large scale than mammalian enzymes.

"There is a long history of people having a lot of problems using enzymes derived from mammals in the kind of **bacterial** expression system that we used. It seemed to us that the **bacterial** enzymes we had been working with are expressed far better than their mammalian counterparts," Wakarchuk said.

Starting with milk sugar, sialic acid and a few other essential ingredients, the researchers used the **fusion** protein to **produce** a carbohydrate called alpha-2,3-sialyllactose, which is present in human breast milk. At first, the authors demonstrated that they could **produce** carbohydrate on the 100-gram scale. Cytel has since begun using the process to ***produce*** the carbohydrate on the 15-kilogram scale. In a field where yields of tens of milligrams of **product** were the norm, ***production*** of kilogram quantities at affordable cost represents a significant advance.

"I think this technology is an important step in the direction of achieving costs consistent with developing carbohydrates as consumer **products**," Paulson said.

Approach Avoids Insolubility Snag, Cuts Costs

The "two-enzymes-in-one" approach gets around the problem of insolubility presented by one of the components, the sialyltransferase. It also reduces costs since it is only necessary to grow one **bacterial** culture instead of two.

Paulson is thinking about the benefits of **fusing** the other two enzymes involved in the **synthesis** of the breast milk carbohydrate.

"A combination of the other two enzymes would **make** sense." Paulson said "When you start thinking about ton scale rather than kilogram scale, that is very important from a cost consideration."

Carbohydrates like that described in the paper require one particular type of nucleotide sugar. To **make** other types of carbohydrates, other building blocks will be needed.

"For a global **synthesis** technology, you need the ability to ***make*** six different nucleotide sugars. Just to expand this idea to other nucleotide sugars would be the first major step. The six sugars would give you more combinations of potential structures, just as amino acids do [in protein ***synthesis***]," Paulson said.

At the moment, Wakarchuk's group has no further plans to work on this particular **fusion** protein but he indicated that his group is constantly looking for new enzymes that will be useful for carbohydrate ***synthesis.*** "Obviously now the idea of making other enzyme ***fusions*** will probably be explored not only by us but by other people," Wakarchuk told BioWorld Today. *

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INDUSTRY CLASS: *BIO Biotechnology; BUSN Any type of business
N. AM. IND. CLASS: *325412 Pharmaceutical Preparation Manufacturing
GEOGRAPHIC TERM: *CC1USA United States
FEATURES: COMPANY

L5 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2004 ACS on STN
RN 9030-18-6 REGISTRY
CN Acetylglucosaminyltransferase, uridine diphosphoacetylglucosamine-chitin (9CI) (CA INDEX NAME)
OTHER NAMES:
CN Chitin oligosaccharide synthase
CN Chitin pentaose synthase
CN **Chitin synthase**
CN Chitin synthetase
CN E.C. 2.4.1.16
CN Gene nodC protein assocd. acetylglucosaminyltransferase
CN Nodulation factor acetylglucosaminyltransferase
DR 106389-20-2
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA, CAPLUS, EMBASE, PROMT, TOXCENTER, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
634 REFERENCES IN FILE CA (1907 TO DATE)
3 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
637 REFERENCES IN FILE CAPLUS (1907 TO DATE)